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(54) Title: HUMAN AND MURINE IL-17D, CYTOKINE RELATED TO INTERLEUKIN-17: DNA AND POLYPEPTIDES			
(57) Abstract DNA encoding IL-17D polypeptides and methods for using the encoded polypeptides are disclosed.			

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HUMAN AND MURINE IL-17D, CYTOKINE RELATED TO INTERLEUKIN-17: DNA AND POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 60/070,886, filed January 9, 1998, which is hereby incorporated by reference.

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BACKGROUND OF THE INVENTION

Field of the Invention

The invention is directed to purified and isolated novel IL-17D polypeptides and fragments thereof, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, and uses thereof.

Description of Related Art

1. IL-17 and IL-17R

Human IL-17 cDNA encodes a cytokine, hIL-17, that induces the production of IL-6, IL-8, and ICAM-1 from human fibroblasts and is homologous with Herpesvirus Saimiri gene 13, HVS13, and mouse cytotoxic T lymphocyte associated antigen 8, CTLA8 (Yao et al., *J. Immunol.* 155:5483-5486, 1995). hIL-17 stimulates epithelial, endothelial, and fibroblastic cells to secrete granulocyte-colony-stimulating factor, as well as prostaglandin E2, and when cultured in the presence of hIL-17, fibroblasts could sustain the proliferation of CD34+ hematopoietic progenitors and their preferential maturation into neutrophils (Fossiez et al., *J. Exp. Med.* 183:2411-2415, 1996). IL-17 synergizes with other proinflammatory cytokines *in vitro*, and induces neutrophilia *in vivo* (Fossiez et al., *Int. Rev. Immunol.* 16:541-551, 1998). IL-17 can stimulate granulopoiesis *in vivo* (Schwarzenberger et al., *J. Immunol.* 161:6383-6389, 1998). hIL-17 increases IL-1 β and TNF α expression from human macrophages (Jovanovic et al., *J. Immunol.* 160:3513-21, 1998). IL-17 has been shown to augment nitric oxide production in osteoarthritis cartilage via NF- κ B activation (Attur et al., *Arthritis. Rheum.* 40:1050-1053, 1997). Stimulation of normal human articular chondrocytes with IL-17 also induces nitric oxide production, increasing iNOS, COX-2, and IL-6 protein expression, and suggesting a signaling pathway involving MAP kinases and NF- κ B (Shalom-Barak et al., *J. Biol. Chem.* 273:27467-27473, 1998).

Mouse, rat, and human IL-17 can recognize the mouse IL-17 receptor (Kennedy et al., *J. Interferon Cytokine Res.* 16:611-617, 1996). hIL-17 receptor, which is 69% identical to the mIL-

17 receptor, was isolated from a human T cell library, and exhibits broad tissue distribution (Yao et al., *Cytokine* 9:794-800, 1997).

Given the important function of IL-17 and IL-17R, there is a need in the art for additional cytokines similar to IL-17. Despite the growing body of knowledge, there is still a need in the art for the identity and function of proteins involved in cellular and immune responses.

2. Protein Identification

In another aspect, the identification of the primary structure, or sequence, of an unknown protein is the culmination of an arduous process of experimentation. In order to identify an unknown protein, the investigator can rely upon a comparison of the unknown protein to known peptides using a variety of techniques known to those skilled in the art. For instance, proteins are routinely analyzed using techniques such as electrophoresis, sedimentation, chromatography, sequencing and mass spectrometry.

In particular, comparison of an unknown protein to polypeptides of known molecular weight allows a determination of the apparent molecular weight of the unknown protein (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)). Protein molecular weight standards are commercially available to assist in the estimation of molecular weights of unknown protein (New England Biolabs Inc. Catalog:130-131, 1995; J. L. Hartley, U.S. Patent No. 5,449,758). However, the molecular weight standards may not correspond closely enough in size to the unknown protein to allow an accurate estimation of apparent molecular weight. The difficulty in estimation of molecular weight is compounded in the case of proteins that are subjected to fragmentation by chemical or enzymatic means, modified by post-translational modification or processing, and/or associated with other proteins in non-covalent complexes.

In addition, the unique nature of the composition of a protein with regard to its specific amino acid constituents results in unique positioning of cleavage sites within the protein. Specific fragmentation of a protein by chemical or enzymatic cleavage results in a unique "peptide fingerprint" (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977; M. Brown et al., *J. Gen. Virol.* 50:309-316, 1980). Consequently, cleavage at specific sites results in reproducible fragmentation of a given protein into peptides of precise molecular weights. Furthermore, these peptides possess unique charge characteristics that determine the isoelectric pH of the peptide. These unique characteristics can be exploited using a variety of

electrophoretic and other techniques (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)).

Fragmentation of proteins is further employed for amino acid composition analysis and protein sequencing (P. Matsudaira, *J. Biol. Chem.* 262:10035-10038, 1987; C. Eckerskorn et al., *Electrophoresis* 1988, 9:830-838, 1988), particularly the production of fragments from proteins with a "blocked" N-terminus. In addition, fragmented proteins can be used for immunization, for affinity selection (R. A. Brown, U.S. Patent No. 5,151,412), for determination of modification sites (e.g. phosphorylation), for generation of active biological compounds (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 300-301 (Prentice Hall, 6d ed. 1991)), and for differentiation of homologous proteins (M. Brown et al., *J. Gen. Virol.* 50:309-316, 1980).

In addition, when a peptide fingerprint of an unknown protein is obtained, it can be compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015, 1993; D. Fenyo et al., *Electrophoresis* 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearchForm.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare these molecular weights to protein molecular weight information stored in databases to assist in determining the identity of the unknown protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in determining the number of fragmented peptides and their molecular weight should result in enhanced likelihood of success in the identification of unknown proteins.

In addition, peptide digests of unknown proteins can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec.* 5:976-989 (1994); M. Mann and M. Wilm, *Anal. Chem.* 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec.* 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site: www.lsbcc.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search

and ProFound programs described above. Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using tandem mass spectrometry.

Thus, there exists a need in the art for polypeptides suitable for use in peptide fragmentation studies, for use in molecular weight measurements, and for use in protein sequencing using tandem mass spectrometry.

SUMMARY OF THE INVENTION

The invention aids in fulfilling these various needs in the art by providing isolated IL-17D nucleic acids and polypeptides encoded by these nucleic acids. Particular embodiments of the invention are directed to an isolated IL-17D nucleic acid molecule comprising the human DNA sequence of SEQ ID NO:1 or the murine DNA sequence of SEQ ID NO:3 and an isolated IL-17D nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, as well as nucleic acid molecules complementary to these sequences. Both single-stranded and double-stranded RNA and DNA nucleic acid molecules are encompassed by the invention, as well as nucleic acid molecules that hybridize to a denatured, double-stranded DNA comprising all or a portion of SEQ ID NO:1 or SEQ ID NO:3. Also encompassed are isolated nucleic acid molecules that are derived by *in vitro* mutagenesis of nucleic acid molecules comprising sequences of SEQ ID NO:1 or SEQ ID NO:3, that are degenerate from nucleic acid molecules comprising sequences of SEQ ID NO:1 or SEQ ID NO:3, and that are allelic variants of DNA of the invention. The invention also encompasses recombinant vectors that direct the expression of these nucleic acid molecules and host cells stably or transiently transformed or transfected with these vectors.

In addition, the invention encompasses methods of using the nucleic acids noted above to identify nucleic acids encoding proteins having IL-17D activity and to study cell signal transduction and the IL-17D system.

The invention also encompasses the use of sense or antisense oligonucleotides from the nucleic acid of SEQ ID NO:1 or SEQ ID NO:3 to inhibit the expression of the polynucleotide encoded by the IL-17D gene.

The invention also encompasses isolated polypeptides and fragments thereof encoded by these nucleic acid molecules including soluble polypeptide portions of SEQ ID NO:2 or SEQ ID NO:4. The invention further encompasses methods for the production of these polypeptides,

including culturing a host cell under conditions promoting expression and recovering the polypeptide from the culture medium. Especially, the expression of these polypeptides in bacteria, yeast, plant, insect, and animal cells is encompassed by the invention.

In general, the polypeptides of the invention can be used to study cellular processes such as immune regulation, cell proliferation, cell death, cell migration, cell-to-cell interaction, and inflammatory responses. In addition, these polypeptides can be used to identify proteins associated with IL-17D ligands and IL-17D receptors.

In addition, the invention includes assays utilizing these polypeptides to screen for potential inhibitors of activity associated with polypeptide counter-structure molecules, and methods of using these polypeptides as therapeutic agents for the treatment of diseases mediated by IL-17D polypeptide counter-structure molecules. Further, methods of using these polypeptides in the design of inhibitors thereof are also an aspect of the invention.

The invention further provides a method for using these polypeptides as molecular weight markers that allow the estimation of the molecular weight of a protein or a fragmented protein, as well as a method for the visualization of the molecular weight markers of the invention thereof using electrophoresis. The invention further encompasses methods for using the polypeptides of the invention as markers for determining the isoelectric point of an unknown protein, as well as controls for establishing the extent of fragmentation of a protein.

Further encompassed by this invention are kits to aid in these determinations.

Further encompassed by this invention is the use of the IL-17D nucleic acid sequences, predicted amino acid sequences of the polypeptide or fragments thereof, or a combination of the predicted amino acid sequences of the polypeptide and fragments thereof, in searching an electronic database to aid in the identification of sample nucleic acids and/or proteins.

Isolated polyclonal or monoclonal antibodies that bind to these polypeptides are also encompassed by the invention, in addition the use of these antibodies to aid in purifying the IL-17D polypeptide.

The invention also encompasses isolated polypeptides encoded by these nucleic acid molecules, including isolated polypeptides having a molecular weight of approximately 20 kD as determined by SDS-PAGE and isolated polypeptides in non-glycosylated form.

The invention further encompasses the fragmented peptides produced from IL-17D polypeptides by chemical or enzymatic treatment. In addition, forms of IL-17D polypeptide molecular weight markers and fragmented peptides thereof, wherein at least one of the sites

necessary for fragmentation by chemical or enzymatic means has been mutated, are an aspect of the invention.

DETAILED DESCRIPTION OF THE INVENTION

5 cDNAs encoding human and mouse IL-17D polypeptides have been isolated and are disclosed in SEQ ID NO:1 and SEQ ID NO:3, respectively. The discovery of the nucleic acids of the invention enables the construction of expression vectors comprising nucleic acid sequences encoding polypeptides; host cells transfected or transformed with the expression vectors; isolated and purified biologically active polypeptides and fragments thereof; the use of 10 the nucleic acids or oligonucleotides thereof as probes to identify nucleic acid encoding proteins having IL-17D activity; the use of single-stranded sense or antisense oligonucleotides from the nucleic acids to inhibit expression of polynucleotide encoded by the IL-17D gene; the use of such polypeptides and soluble fragments to the use of such polypeptides and fragmented peptides as molecular weight markers; the use of such polypeptides and fragmented peptides as controls 15 for peptide fragmentation, and kits comprising these reagents; the use of such polypeptides and fragments thereof to generate antibodies; and the use of antibodies to purify the IL-17D polypeptide.

IL-17D DNA was originally seen as EST clones in the public databases, specifically GB accession # 1505551 (from a human fetal heart library) and GB accession # W83241 (from a 20 mouse embryo library). The human and mouse ESTs were re-sequenced and found to contain an amino terminal hydrophobic sequence that could function as a signal peptide (amino acids 1-20 of SEQ ID NO:1 and SEQ ID NO:2), no hydrophobic transmembrane-like regions, and a stop codon. The predicted topology of IL-17 polypeptides is consistent with a secreted protein. The cDNA sequence of the human and mouse IL-17D DNA is given in SEQ ID NO:1 and SEQ ID 25 NO:3, respectively.

The nucleotide sequence of human IL-17D DNA is:

ATGGACTGGCCTCACAACTGCTGTTTCTTCTTACCATTTCATCTTCCT
 GGGGCTGGGCCAGCCCAGGAGCCCCAAAAGCAAGAGGAAGGGGCAAGGGC
 GGCCTGGGCCCCCTGGCCCCCTGACCAGGTGCCACTGGACCTGGTG
 30 TCACGGATGAAACCGTATGCCCCGATGGAGGAGTATGAGAGGAACATCGA
 GGAGATGGTGGCCCAGCTGAGGAACAGCTCAGAGCTGGCCCAGAGAAAGT
 GTGAGGTCAACTTGACAGCTGTGGATGTCCAACAAGAGGAGCCTGTCTCCC

TGGGGCTACAGCATCAACCACGACCCCAGCCGTATCCCCGTGGACCTGCC
GGAGGCACGGTGCCTGTGTCTGGGCTGTGTGAACCCCTTCACCATGCAGG
AGGACCGCAGCATGGTGAGCGTGCCGGTGTTAGCCAGGTTCTGTGCGC
CGCCGCCTCTGCCCCGCCACCGCCCCGCACAGGGCCTTGCCGCCAGCGCGC
5 AGTCATGGAGACCATCGCTGTGGGCTGCACCTGCATCTTC (SEQ ID NO:1).

The nucleotide sequence of mouse IL-17D DNA is:

ATGGACTGGCCGCACAGCCTGCTCTTCCTCCTGGCCATCTCCATCTTCCT
GGCGCCAAGCCACCCCCGGAACACCAAAGGCAAAAGAAAAGGGCAAGGGA
GGCCCAGTCCCTTGCCCCCTGGGCCTCATCAGGTGCCGCTGGACCTGGTG
10 TCTCGAGTAAAGCCCTACGCTCGAATGGAAGAGTATGAGCGGAACCTTGG
GGAGATGGTGGCCCAGCTGAGGAACAGCTCCGAGCCAGCCAAGAAGAAAT
GTGAAGTCAATCTACAGCTGTGGTTGTCCAACAAGAGGAGCCTGTCCCCA
TGGGGCTACAGCATCAACCACGACCCCAGCCGCATCCCTGCGGACTTGCC
CGAGGCGCGGTGCCTATGTTTGGGTTGCGTGAATCCCTTCACCATGCAGG
15 AGGACCGTAGCATGGTGAGCGTGCCAGTGTTAGCCAGGTGCCGGTGCGC
CGCCGCCTCTGTCTCAACCTCCTCGCCCTGGGCCCTGCCGCCAGCGTGT
CGTCATGGAGACCATCGCTGTGGGTTGCACCTGCATCTTC (SEQ ID NO:3).

SEQ ID NO:1 and SEQ ID NO:3 encode human IL-17D polypeptide (SEQ ID NO:2) and mouse IL-17D polypeptide (SEQ ID NO:4), respectively.

20 The amino acid sequence of human IL-17D polypeptide is:

MDWPHNLLFLLTISIFLGLGQPRSPKSKRKGQGRPGPLAPGPHQVPLDLV
SRMKPYARMEEYERNIEEMVAQLRNSSELAQRKCEVNLQLWMSNKRSLSP
WGYSINHDPRI PVDLPEARCLCLGCVNPFTMQEDRSMVSPVFSQVPVR
RRLCPPPPRTGPCRQRAVMEIIVGCTCIF (SEQ ID NO:2).

25 The amino acid sequence of mouse IL-17D polypeptide is:

MDWPHSLLFLLAISIFLAPSHPRNTKGKRKGQGRPSPLAPGPHQVPLDLV
SRVKPYARMEEYERNLGEMVAQLRNSSEPAKKKCEVNLQLWLSNKRSLSP
WGYSINHDPRI PADLPEARCLCLGCVNPFTMQEDRSMVSPVFSQVPVR
RRLCPQP RPGRQVRVMEIIVGCTCIF (SEQ ID NO:4).

30 Both human and mouse IL-17D polypeptides are significantly related to mouse and human IL-17 polypeptides, and to each other. Human IL-17D is 34% identical to human IL-17

and mouse IL-17D is 35% identical to mouse IL-17. Human and mouse IL-17D are 85% identical.

Since IL-17D polypeptides encode a cytokine, which is related to IL-17 and can be secreted from cells, IL-17D polypeptides can bind to cytokine receptors that are similar to IL-17R, as well as other cytokine receptors. The observed homology between IL-17D and IL-17 suggests that IL-17D polypeptide is capable of signaling through cytokine receptors. There also exists the possibility that one or more of IL-17D polypeptides are membrane bound by means of a GPI linkage. A GPI-linked IL-17D polypeptide may serve to transmit an intracellular signal following activation. In addition, similar to IL-17, IL-17D polypeptides can induce the production of other cytokines, such as IL-1, IL-6, and IL-8 from fibroblasts and epithelial cells.

Both mouse and human IL-17D polypeptides were expressed as chimeric, recombinant proteins having either an "Fc" or polyhistidine tag. These proteins were expressed in mammalian cells in sufficient quantities to allow purification.

Monoclonal antibodies were generated against human IL-17D polypeptide. Mice were injected with his-flag-human IL-17D polypeptide, and sera were screened for binding to the his-flag-human IL-17D polypeptide in an ELISA and by radioimmunoprecipitation. Four hybridomas producing monoclonal antibodies (designated M491, M492, M493, and M494) were isolated and purified. These monoclonal antibodies will immunoprecipitate radiolabeled his-flag-human IL-17D polypeptide, but will not block the binding of his-flag-human IL-17D polypeptide or flag-Fc-human IL-17D polypeptide to cell lines as assessed by FACS.

Flow cytometric analysis was performed on primary cells and cell lines using mouse or human IL-17D.Fc proteins. Using human IL-17D.Fc, significant binding was seen to human foreskin fibroblasts and human lung epithelial line cells (WI26); however Scatchard analysis indicated that this binding was non-specific. Binding to other adherent cell lines HT1080T, CV-1, COS, 293EBNA, TMOF, and SK-Hep is also thought to be non-specific. Specific binding of IL-17D polypeptide to suspension cell lines EL40.5, TF-1, S49.1, and K299 was demonstrated. Using mouse IL-17D.Fc, significant binding was seen to the B220+ population of mouse splenocytes (predominantly B220+/IgM+/IgD+), indicating binding of IL-17 polypeptides to B cells. Since IL-17D polypeptides bind to B cells, it is likely that these polypeptides can regulate B cell differentiation and proliferation.

Experiments indicate that IL-17D interacts with the cloned IL-17R with low affinity. Since the mouse IL-17D.Fc molecule binds well to the B220+ population of IL-17R knock out

mice, an additional receptor can be used by IL-17D polypeptides. Since IL-17D polypeptides bind to B cells, it is likely that these polypeptides can be used for targeting compounds to B cells and B cell tumors, and for specific selection of B cell populations.

Cell/tissue distribution of IL-17D was examined by RT-PCR. RNA expression was detected in A549 and THP-1 cells, but not in primary peripheral blood T cells, primary NK cells, primary neutrophils, primary monocytes, foreskin fibroblasts, Jurkat cells, and JM-1 cells.

NUCLEIC ACID MOLECULES

In a particular embodiment, the invention relates to certain isolated nucleotide sequences that are free from contaminating endogenous material. A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

Nucleic acid molecules of the invention include DNA in both single-stranded and double-stranded form, as well as the RNA complement thereof. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. Genomic DNA may be isolated by conventional techniques, e.g., using the cDNA of SEQ ID NO:1 or SEQ ID NO:3, or a suitable fragment thereof, as a probe.

The DNA molecules of the invention include full length genes as well as polynucleotides and fragments thereof. The full length gene may include the N-terminal signal peptide. Other embodiments include DNA encoding a soluble form, e.g., encoding the extracellular domain of the protein, either with or without the signal peptide.

The nucleic acids of the invention are preferentially derived from human and murine sources, but the invention includes those derived from sources, as well.

Preferred Sequences

Particularly preferred nucleotide sequences of the invention are SEQ ID NO:1 and SEQ ID NO:3. The sequences of amino acids encoded by the DNA of SEQ ID NO:1 and SEQ ID NO:3 are shown in SEQ ID NO:2 and SEQ ID NO:4.

Additional Sequences

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NO:1 or SEQ ID NO:3, and still encode a polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Such variant DNA sequences can result from silent mutations (*e.g.*, occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA sequences encoding polypeptides of the invention, selected from: (a) DNA derived from the coding region of a native mammalian IL-17D gene; (b) DNA comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3; (c) DNA encoding the polypeptides of SEQ ID NO:2 or SEQ ID NO:4; (d) DNA capable of hybridization to a DNA of (b) or (c) under conditions of moderate stringency and which encodes polypeptides of the invention; (e) DNA capable of hybridization to a DNA of (b) or (c) under conditions of high stringency and which encodes polypeptides of the invention, and (f) DNA which is degenerate as a result of the genetic code to a DNA defined in (b), (c), (d) or (e) and which encode polypeptides of the invention. Of course, polypeptides encoded by such DNA sequences are encompassed by the invention. In addition, IL-17D polypeptides encoded by DNA derived from other mammalian species, wherein the DNA will hybridize to the complement of the DNA of SEQ ID NO:1 or SEQ ID NO:3, are also encompassed.

As used herein, conditions of moderate stringency can be readily determined by those having ordinary skill in the art based on, for example, the length of the DNA. The basic conditions are set forth by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), and include use of a prewashing solution for the nitrocellulose filters 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50% formamide, 6X SSC at about 42°C (or other similar hybridization solution, such as Stark's solution, in about 50% formamide at about 42°C), and

washing conditions of about 60°C, 0.5X SSC, 0.1% SDS. Conditions of high stringency can also be readily determined by the skilled artisan based on, for example, the length of the DNA. Generally, such conditions are defined as hybridization conditions as above, and with washing at approximately 68°C, 0.2X SSC, 0.1% SDS. The skilled artisan will recognize that the
5 temperature and wash solution salt concentration can be adjusted as necessary according to factors such as the length of the probe.

Also included as an embodiment of the invention is DNA encoding polypeptide fragments and polypeptides comprising inactivated N-glycosylation site(s), inactivated protease processing site(s), or conservative amino acid substitution(s), as described below.

10 In another embodiment, the nucleic acid molecules of the invention also comprise nucleotide sequences that are at least 80% identical to a native sequence. Also contemplated are embodiments in which a nucleic acid molecule comprises a sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to a native sequence.

15 The percent identity may be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two nucleic acid sequences can be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program
20 include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3)
25 no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention also provides isolated nucleic acids useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding a IL-17D polypeptide, or desired fragment thereof may
30 be subcloned into an expression vector for production of the polypeptide or fragment. The DNA sequence advantageously is fused to a sequence encoding a suitable leader or signal peptide. Alternatively, the desired fragment may be chemically synthesized using known techniques.

DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. If necessary, oligonucleotides that reconstruct the 5' or 3' terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well-known polymerase chain reaction (PCR) procedure also may be employed to isolate and amplify a DNA sequence encoding a desired protein fragment. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified DNA fragment into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego (1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications*, Innis et al., eds., Academic Press, Inc. (1990).

POLYPEPTIDES AND FRAGMENTS THEREOF

The invention encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA technology. For example, DNAs encoding IL-17D polypeptides can be derived from SEQ ID NO:1 or SEQ ID NO:3 by *in vitro* mutagenesis, which includes site-directed mutagenesis, random mutagenesis, and *in vitro* nucleic acid synthesis. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

Polypeptides and Fragments Thereof

The polypeptides of the invention include full length proteins encoded by the nucleic acid sequences set forth above. Particularly preferred polypeptides comprise the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, with particularly preferred fragments comprising amino acids 21-180 of SEQ ID NO:2 and amino acids 21-180 of SEQ ID NO:4.

Soluble polypeptides are capable of being secreted from the cells in which they are expressed. In general, soluble polypeptides may be identified (and distinguished from non-soluble membrane-bound counterparts) by separating intact cells which express the desired

polypeptide from the culture medium, *e.g.*, by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the protein.

5 Soluble polypeptides thus include, but are not limited to, polypeptides lacking the signal sequence. Such polypeptides comprise amino acids 21-180 of SEQ ID NO:2 or SEQ ID NO:4.

In general, the use of soluble forms is advantageous for certain applications. Purification of the polypeptides from recombinant host cells is facilitated, since the soluble polypeptides are secreted from the cells. Further, soluble polypeptides are generally more suitable for intravenous
10 administration.

The invention also provides polypeptides and fragments that retain a desired biological activity. Particular embodiments are directed to polypeptide fragments that retain the ability to bind IL-17D counter-structures. Such a fragment may be a soluble polypeptide, as described above. In another embodiment, the polypeptides and fragments advantageously include regions
15 that are conserved in the IL-17D family as described above.

Also provided herein are polypeptide fragments comprising a varying number of contiguous amino acids ranging from 6 to 100. In preferred embodiments the fragments comprise at least 20, or at least 30, contiguous amino acids of the sequence of SEQ ID NO:2 or SEQ ID NO:4. Polypeptide fragments also may be employed as immunogens, in generating
20 antibodies.

Variants

Naturally occurring variants as well as derived variants of the polypeptides and fragments are provided herein.

25 An "IL-17D variant" as referred to herein means a polypeptide substantially homologous to native IL-17D polypeptide, but which has an amino acid sequence different from that of native IL-17D polypeptide (human, murine or other mammalian species) because of one or more deletions, insertions, or substitutions. The variant has an amino acid sequence that preferably is at least 80% identical to a native IL-17D polypeptide amino acid sequence, most preferably at
30 least 90% identical. The percent identity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics

Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math* 2:482, 1981). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for
5 nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

10 Variants also include embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof. Percent identity may be determined as above. Alternatively, the percent identity of two protein sequences can be determined by comparing sequence information using the GAP
15 computer program, based on the algorithm of Needleman and Wunsch (*J. Mol. Bio.* 48:443, 1970) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a scoring matrix, blosum62, as described by Henikoff and Henikoff (*Proc. Natl. Acad. Sci. USA* 89:10915, 1992); (2) a gap weight of 12; (3) a gap length weight of 4; and (4) no penalty for end gaps. Other programs
20 used by one skilled in the art of sequence comparison may also be used.

The variants of the invention include, for example, those that result from alternate mRNA splicing events or from proteolytic cleavage. Alternate splicing of mRNA may, for example, yield a truncated but biologically active protein, such as a naturally occurring soluble form of the protein. Variations attributable to proteolysis include, for example, differences in the N- or C-
25 termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the protein (generally from 1-5 terminal amino acids). Proteins in which differences in amino acid sequence are attributable to genetic polymorphism (allelic variation among individuals producing the protein) are also contemplated herein.

As stated above, the invention provides isolated and purified, or homogeneous, IL-17D
30 polypeptides, both recombinant and non-recombinant. Variants and derivatives of native IL-17D proteins that retain the desired biological activity can be obtained by mutations of nucleotide sequences coding for native IL-17D polypeptides. Alterations of the native amino acid sequence

can be accomplished by any of a number of conventional methods. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene, wherein predetermined codons can be altered by substitution, deletion, or insertion. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985); Kunkel et al. (*Methods in Enzymol.* 154:367, 1987); and U.S. Patent Nos. 4,518,584 and 4,737,462, all of which are incorporated by reference.

IL-17D polypeptides can be modified to create IL-17D derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, polyethylene glycol (PEG) groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of IL-17D polypeptides can be prepared by linking the chemical moieties to functional groups on IL-17D amino acid side chains or at the N-terminus or C-terminus of a IL-17D polypeptide or the extracellular domain thereof. Other derivatives of IL-17D polypeptides within the scope of this invention include covalent or aggregative conjugates of IL-17D polypeptides or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugate can comprise a signal or leader polypeptide sequence (e.g. the α -factor leader of *Saccharomyces*) at the N-terminus of a IL-17D polypeptide. The signal or leader peptide co-translationally or post-translationally directs transfer of the conjugate from its site of synthesis to a site inside or outside of the cell membrane or cell wall.

Conjugates comprising diagnostic (detectable) or therapeutic agents attached thereto are contemplated herein, as discussed in more detail below.

Other derivatives include covalent or aggregative conjugates of the polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with oligomers. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in

U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG[®] peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Lys, which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11
5 produces a monoclonal antibody that binds the FLAG[®] peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG[®] peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

10 Among the variant polypeptides provided herein are variants of native polypeptides that retain the native biological activity or the substantial equivalent thereof. One example is a variant that binds with essentially the same binding affinity as does the native form. Binding affinity can be measured by conventional procedures, *e.g.*, as described in U.S. Patent No. 5,512,457 and as set forth below.

15 Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.

20 A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other
25 conservative substitutions, *e.g.*, involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, the DNAs of the invention include variants that differ from a native DNA sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide.

30 The invention further includes polypeptides of the invention with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems (*e.g.*, COS-1 or COS-7 cells) can be similar to or significantly different from a native

polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of polypeptides of the invention in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Further, a given preparation may include multiple differentially glycosylated species of the protein. Glycosyl groups can be removed
5 through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated polypeptides of the invention can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

Correspondingly, similar DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences
10 are encompassed by the invention. For example, N-glycosylation sites can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions, or deletions to the nucleotide sequence encoding these
15 triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Alternatively, the Ser or Thr can be replaced with another amino acid, such as Ala. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846,
20 hereby incorporated by reference.

In another example of variants, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation.

Other variants are prepared by modification of adjacent dibasic amino acid residues, to
25 enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these
30 adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Oligomers

Encompassed by the invention are oligomers or fusion proteins that contain IL-17D polypeptides. Such oligomers may be in the form of covalently-linked or non-covalently-linked multimers, including dimers, trimers, or higher oligomers. As noted above, preferred polypeptides are soluble and thus these oligomers may comprise soluble polypeptides. In one aspect of the invention, the oligomers maintain the binding ability of the polypeptide components and provide therefor, bivalent, trivalent, etc., binding sites.

One embodiment of the invention is directed to oligomers comprising multiple polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of the polypeptides attached thereto, as described in more detail below.

Immunoglobulin-based Oligomers

As one alternative, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi et al. (*PNAS USA* 88:10535, 1991); Byrn et al. (*Nature* 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11, 1992).

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a polypeptide of the invention to an Fc polypeptide derived from an antibody. A gene fusion encoding the polypeptide/Fc fusion protein is inserted into an appropriate expression vector. Polypeptide/Fc fusion proteins are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent molecules.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides made up of the Fc region of an antibody comprising any or all of the CH domains of the Fc region. Truncated forms of such polypeptides containing the hinge region that

promotes dimerization are also included. Preferred polypeptides comprise an Fc polypeptide derived from a human IgG1 antibody.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum et al., (*EMBO J.* 13:3992-4001, 1994) incorporated herein by reference. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

The above-described fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

In other embodiments, the polypeptides of the invention may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an oligomer with as many as four IL-17D extracellular regions.

Peptide-linker Based Oligomers

Alternatively, the oligomer is a fusion protein comprising multiple polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences of the invention, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between the sequences. In particular embodiments, a fusion protein comprises from two to four soluble IL-17D polypeptides, separated by peptide linkers.

Leucine-Zippers

Another method for preparing the oligomers of the invention involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in

which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize.

5 The zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, often with four or five leucine residues interspersed with other amino acids. Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*,
10 also exhibit zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise zipper domains that preferentially form heterodimer (O'Shea et al., *Science* 245:646, 1989, Turner and Tjian, *Science* 243:1689, 1989). The zipper domain is necessary for biological activity (DNA binding) in these proteins.

15 The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess zipper domains (Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the zipper
20 domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al, *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

 Zipper domains fold as short, parallel coiled coils (O'Shea et al., *Science* 254:539; 1991).
25 The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (*J. Mol. Biol.* 98:293; 1975), in which residues *a* and *d* are generally hydrophobic residues, with *d* being a leucine, which line up on the same face of a
30 helix. Oppositely-charged residues commonly occur at positions *g* and *e*. Thus, in a parallel coiled coil formed from two helical zipper domains, the "knobs" formed by the hydrophobic side

chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The residues at position *d* (often leucine) contribute large hydrophobic stabilization energies, and are important for oligomer formation (Krystek: et al., *Int. J. Peptide Res.* 38:229, 1991). Lovejoy et al. (*Science* 259:1288, 1993) recently reported the synthesis of a triple-stranded α -helical bundle in which the helices run up-up-down. Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils. Further discussion of the structure of leucine zippers is found in Harbury et al. (*Science* 262:1401, 26 November 1993).

Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al. (*FEBS Letters* 344:191, 1994), hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al. (*Semin. Immunol.* 6:267-278, 1994). Recombinant fusion proteins comprising a soluble polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomer that forms is recovered from the culture supernatant.

Certain leucine zipper moieties preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, 1994) and in U.S. Patent 5,716,805, hereby incorporated by reference in their entirety. This lung SPD-derived leucine zipper peptide comprises the amino acid sequence Pro Asp Val Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His Leu Gln Ala Ala Phe Ser Gln Tyr.

Another example of a leucine zipper that promotes trimerization is a peptide comprising the amino acid sequence Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg, as described in U.S. Patent 5,716,805. In one alternative embodiment, an N-terminal Asp residue is added; in another, the peptide lacks the N-terminal Arg residue.

Fragments of the foregoing zipper peptides that retain the property of promoting oligomerization may be employed as well. Examples of such fragments include, but are not limited to, peptides lacking one or two of the N-terminal or C-terminal residues presented in the

foregoing amino acid sequences. Leucine zippers may be derived from naturally occurring leucine zipper peptides, e.g., *via* conservative substitution(s) in the native amino acid sequence, wherein the peptide's ability to promote oligomerization is retained.

Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric IL-17D. Alternatively, synthetic peptides that promote oligomerization may be employed. In particular embodiments, leucine residues in a leucine zipper moiety are replaced by isoleucine residues. Such peptides comprising isoleucine may be referred to as isoleucine zippers, but are encompassed by the term "leucine zippers" as employed herein.

PRODUCTION OF POLYPEPTIDES AND FRAGMENTS THEREOF

Expression, isolation and purification of the polypeptides and fragments of the invention may be accomplished by any suitable technique, including but not limited to the following:

Expression Systems

The present invention also provides recombinant cloning and expression vectors containing DNA, as well as host cell containing the recombinant vectors. Expression vectors comprising DNA may be used to prepare the polypeptides or fragments of the invention encoded by the DNA. A method for producing polypeptides comprises culturing host cells transformed with a recombinant expression vector encoding the polypeptide, under conditions that promote expression of the polypeptide, then recovering the expressed polypeptides from the culture. The skilled artisan will recognize that the procedure for purifying the expressed polypeptides will vary according to such factors as the type of host cells employed, and whether the polypeptide is membrane-bound or a soluble form that is secreted from the host cell.

Any suitable expression system may be employed. The vectors include a DNA encoding a polypeptide or fragment of the invention, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA sequence. Thus, a promoter nucleotide sequence is operably linked to a DNA sequence if the promoter nucleotide sequence controls the transcription of the DNA sequence. An origin of replication that confers

the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the nucleic acid sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide upon secretion of polypeptide from the cell.

The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved may differ from that predicted by computer program, and may vary according to such factors as the type of host cells employed in expressing a recombinant polypeptide. A protein preparation may include a mixture of protein molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site. Particular embodiments of mature proteins provided herein include, but are not limited to, proteins having the residue at position 1 or 21 of SEQ ID NO:2 or SEQ ID NO:4 as the N-terminal amino acid.

Suitable host cells for expression of polypeptides include prokaryotes, yeast or higher eukaryotic cells. Mammalian or insect cells are generally preferred for use as host cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al. *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotic Systems

Prokaryotes include gram-negative or gram-positive organisms. Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as *E. coli*, a polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those
5 derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. An appropriate promoter and a DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec,
10 Madison, WI, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β -lactamase (penicillinase), lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EP-A-36776) and tac promoter (Maniatis,
15 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λP_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λP_L promoter include plasmid pHUB2 (resident in *E. coli* strain JMB9, ATCC 37092) and pPLc28 (resident in *E. coli* RR1, ATCC
20 53082).

IL-17D DNA may be cloned in-frame into the multiple cloning site of an ordinary bacterial expression vector. Ideally the vector would contain an inducible promoter upstream of the cloning site, such that addition of an inducer leads to high-level production of the recombinant protein at a time of the investigator's choosing. For some proteins, expression
25 levels may be boosted by incorporation of codons encoding a fusion partner (such as hexahistidine) between the promoter and the gene of interest. The resulting "expression plasmid" may be propagated in a variety of strains of *E. coli*.

For expression of the recombinant protein, the bacterial cells are propagated in growth medium until reaching a pre-determined optical density. Expression of the recombinant protein
30 is then induced, e.g. by addition of IPTG (isopropyl-b-D-thiogalactopyranoside), which activates expression of proteins from plasmids containing a lac operator/promoter. After induction

(typically for 1-4 hours), the cells are harvested by pelleting in a centrifuge, e.g. at 5,000 x G for 20 minutes at 4°C.

For recovery of the expressed protein, the pelleted cells may be resuspended in ten volumes of 50 mM Tris-HCl (pH 8)/1 M NaCl and then passed two or three times through a French press. Most highly expressed recombinant proteins form insoluble aggregates known as inclusion bodies. Inclusion bodies can be purified away from the soluble proteins by pelleting in a centrifuge at 5,000 x G for 20 minutes, 4°C. The inclusion body pellet is washed with 50 mM Tris-HCl (pH 8)/1% Triton X-100 and then dissolved in 50 mM Tris-HCl (pH 8)/8 M urea/ 0.1 M DTTf. Any material that cannot be dissolved is removed by centrifugation (10,000 x G for 20 minutes, 20°C). The protein of interest will, in most cases, be the most abundant protein in the resulting clarified supernatant. This protein may be "refolded" into the active conformation by dialysis against 50 mM Tris-HCl (pH 8)/5 mM CaCl₂/5 mM Zn(OAc)₂/1 mM GSSG/0.1 mM GSH. After refolding, purification can be carried out by a variety of chromatographic methods, such as ion exchange or gel filtration. In some protocols, initial purification may be carried out before refolding. As an example, hexahistidine-tagged fusion proteins may be partially purified on immobilized Nickel.

While the preceding purification and refolding procedure assumes that the protein is best recovered from inclusion bodies, those skilled in the art of protein purification will appreciate that many recombinant proteins are best purified out of the soluble fraction of cell lysates. In these cases, refolding is often not required, and purification by standard chromatographic methods can be carried out directly.

Yeast Systems

Alternatively, the polypeptides may be expressed in yeast host cells, preferably from the *Saccharomyces* genus (e.g., *S. cerevisiae*). Other genera of yeast, such as *Pichia* or *Kluyveromyces*, may also be employed. Yeast vectors will often contain an origin of replication sequence from a 2μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase,

hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phospho-glucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657. Another alternative is the glucose-repressible
5 ADH2 promoter described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* may be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

The yeast α -factor leader sequence may be employed to direct secretion of the
10 polypeptide. The α -factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. See, e.g., Kurjan et al., *Cell* 30:933, 1982 and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to those of skill in the art. A leader sequence may be modified near its 3' end to contain one or more restriction sites. This will
15 facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978. The Hinnen et al. protocol selects for Trp⁺ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 mg/ml adenine and
20 20 mg/ml uracil.

Yeast host cells transformed by vectors containing an ADH2 promoter sequence may be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 mg/ml adenine and 80 mg/ml uracil. Derepression of the ADH2 promoter occurs when glucose is
25 exhausted from the medium.

Mammalian or Insect Systems

Mammalian or insect host cell culture systems also may be employed to express recombinant polypeptides. Baculovirus systems for production of heterologous proteins in
30 insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also may be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al.,

Cell 23:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

5 Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture*, 1990, pp. 15-69). Additional protocols using commercially available reagents, such as Lipofectamine lipid reagent (Gibco/BRL) or Lipofectamine-Plus lipid reagent, can be used to transfect cells (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1987). In addition, electroporation can be used to transfect mammalian
10 cells using conventional procedures, such as those in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman et al., *Meth. in Enzymology* 185:487-511, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A
15 suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be
20 incorporated into an expression vector include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

 Transcriptional and translational control sequences for mammalian host cell expression vectors can be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human
25 cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers et al.,
30 *Nature* 273:113, 1978; Kaufman, *Meth. in Enzymology*, 1990). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris et al., *Animal Cell Technology*, 1997, pp. 529-534 and PCT Application WO 97/25420) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras et al., *J. Biol. Chem.* 257:13475-13491, 1982). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, *Current Opinion in Genetics and Development* 3:295-300, 1993; Ramesh et al., *Nucleic Acids Research* 24:2697-2700, 1996). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for a selectable marker (e.g. DHFR) has been shown to improve transfectability of the host and expression of the heterologous cDNA (Kaufman, *Meth. in Enzymology*, 1990). Exemplary expression vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser et al., *Biotechniques* 22:150-161, 1997, and p2A5I described by Morris et al., *Animal Cell Technology*, 1997, pp. 529-534.

A useful high expression vector, pCAVNOT, has been described by Mosley et al., *Cell* 59:335-348, 1989. Other expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature* 312:768, 1984, has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in WO 91/18982, incorporated by reference herein. In yet another alternative, the vectors can be derived from retroviruses.

Additional useful expression vectors, pFLAG[®] and pDC311, can also be used. FLAG[®] technology is centered on the fusion of a low molecular weight (1kD), hydrophilic, FLAG[®] marker peptide to the N-terminus of a recombinant protein expressed by pFLAG[®] expression vectors. pDC311 is another specialized vector used for expressing proteins in CHO cells. pDC311 is characterized by a bicistronic sequence containing the gene of interest and a dihydrofolate reductase (DHFR) gene with an internal ribosome binding site for DHFR translation, an expression augmenting sequence element (EASE), the human CMV promoter, a tripartite leader sequence, and a polyadenylation site.

Regarding signal peptides that may be employed, the native signal peptide may be replaced by a heterologous signal peptide or leader sequence, if desired. The choice of signal

peptide or leader may depend on factors such as the type of host cells in which the recombinant polypeptide is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for interleukin-2 receptor described in
5 Cosman et al., *Nature* 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

Purification

10 The invention also includes methods of isolating and purifying the polypeptides and fragments thereof. An isolated and purified IL-17D polypeptide according to the invention can be produced by recombinant expression systems as described above or purified from naturally occurring cells. IL-17D polypeptide can be substantially purified, as indicated by a single protein band upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). One
15 process for producing IL-17D comprises culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes IL-17D polypeptide under conditions sufficient to promote expression of IL-17D. IL-17D polypeptide is then recovered from culture medium or cell extracts, depending upon the expression system employed.

Isolation and Purification

20 The expression "isolated and purified" as used herein means that IL-17D is essentially free of association with other DNA, proteins, or polypeptides, for example, as a purification product of recombinant host cell culture or as a purified product from a non-recombinant source. The term "substantially purified" as used herein refers to a mixture that contains IL-17D and is
25 essentially free of association with other DNA, proteins, or polypeptides, but for the presence of known DNA or proteins that can be removed using a specific antibody, and which substantially purified IL-17D proteins retain biological activity. The term "purified IL-17D" refers to either the "isolated and purified" form of IL-17D or the "substantially purified" form of IL-17D, as both are described herein.

30 The term "biologically active" as it refers to IL-17D protein means that the IL-17D protein is capable of associating with IL-17D counterstructures or being co-immunoprecipitated with IL-17D counterstructures using an antibody to the IL-17D counterstructure.

In one preferred embodiment, the purification of recombinant polypeptides or fragments can be accomplished using fusions of polypeptides or fragments of the invention to another polypeptide to aid in the purification of polypeptides or fragments of the invention. Such fusion partners can include the poly-His or other antigenic identification peptides described above as well as the Fc moieties described previously.

With respect to any type of host cell, as is known to the skilled artisan, procedures for purifying a recombinant polypeptide or fragment will vary according to such factors as the type of host cells employed and whether or not the recombinant polypeptide or fragment is secreted into the culture medium.

In general, the recombinant polypeptide or fragment can be isolated from the host cells if not secreted, or from the medium or supernatant if soluble and secreted, followed by one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification or size exclusion chromatography steps. As to specific ways to accomplish these steps, the culture medium first can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In addition, a chromatofocusing step can be employed. Alternatively, a hydrophobic interaction chromatography step can be employed. Suitable matrices can be phenyl or octyl moieties bound to resins. In addition, affinity chromatography with a matrix which selectively binds the recombinant protein can be employed. Examples of such resins employed are lectin columns, dye columns, and metal-chelating columns. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel or polymer resin having pendant methyl, octyl, octyldecyl or other aliphatic groups) can be employed to further purify the polypeptides. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide an isolated and purified recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion chromatography steps. Finally, RP-HPLC
5 can be employed for final purification steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Transformed yeast host cells are preferably employed to express IL-17D as a secreted polypeptide in order to simplify purification. Secreted recombinant polypeptide from a yeast
10 host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

It is also possible to utilize an affinity column comprising a polypeptide-binding protein of the invention, such as a monoclonal antibody generated against polypeptides of the invention,
15 to affinity-purify expressed polypeptides. These polypeptides can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized, or be competitively removed using the naturally occurring substrate of the affinity moiety, such as a polypeptide derived from the invention.

In this aspect of the invention, polypeptide-binding proteins, such as the anti-polypeptide
20 antibodies of the invention or other proteins that may interact with the polypeptide of the invention, can be bound to a solid phase support such as a column chromatography matrix or a similar substrate suitable for identifying, separating, or purifying cells that express polypeptides of the invention on their surface. Adherence of polypeptide-binding proteins of the invention to
25 a solid phase contacting surface can be accomplished by any means, for example, magnetic microspheres can be coated with these polypeptide-binding proteins and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid phase that has such polypeptide-binding proteins thereon. Cells having polypeptides of the invention on their surface bind to the fixed polypeptide-binding protein and unbound cells then are washed
30 away. This affinity-binding method is useful for purifying, screening, or separating such polypeptide-expressing cells from solution. Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such

enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner.

Alternatively, mixtures of cells suspected of containing polypeptide-expressing cells of the invention first can be incubated with a biotinylated polypeptide-binding protein of the invention. Incubation periods are typically at least one hour in duration to ensure sufficient binding to polypeptides of the invention. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. See Berenson, et al. *J. Cell. Biochem.*, 10D:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

In the methods described above, suitable IL-17D-binding polypeptides are anti-IL-17D antibodies and other proteins that are capable of high-affinity binding of IL-17D. A preferred IL-17D-binding protein is an anti-IL-17D monoclonal antibody.

The desired degree of purity depends on the intended use of the protein. A relatively high degree of purity is desired when the polypeptide is to be administered *in vivo*, for example. In such a case, the polypeptides are purified such that no protein bands corresponding to other proteins are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to the polypeptide may be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. Most preferably, the polypeptide of the invention is purified to substantial homogeneity, as indicated by a single protein band upon analysis by SDS-PAGE. The protein band may be visualized by silver staining, Coomassie blue staining, or (if the protein is radiolabeled) by autoradiography.

Assays

The purified polypeptides of the invention (including proteins, polypeptides, fragments, variants, oligomers, and other forms) may be tested for the ability to bind a IL-17D counter-structure molecule in any suitable assay, such as a conventional binding assay. To illustrate, the polypeptide may be labeled with a detectable reagent (e.g., a radionuclide, chromophore, enzyme that catalyzes a colorimetric or fluorometric reaction, and the like). The labeled polypeptide is contacted with cells expressing a IL-17D counter-structure molecule. The cells then are washed

to remove unbound labeled polypeptide, and the presence of cell-bound label is determined by a suitable technique, chosen according to the nature of the label.

One example of a binding assay procedure is as follows. A recombinant expression vector containing IL-17D counter-structure cDNA is constructed, for example, fusing the extracellular domain of a IL-17D counter-structure molecule to the IgG-I Fc (mutein form) as previously described for OX40-Fc (Baum et al., *EMBO J.* 13:3992-4001, 1994). CV1-EBNA-1 cells in 10 cm² dishes are transfected with the recombinant expression vector. CV-1/EBNA-1 cells (ATCC CRL 10478) constitutively express EBV nuclear antigen-1 driven from the CMV immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al. (*EMBO J.* 10:2821, 1991).

The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4 x 10⁴ cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated for 1 hour at 37°C with various concentrations of, for example, a soluble polypeptide/Fc fusion protein made as set forth above. Cells then are washed and incubated with a constant saturating concentration of a ¹²⁵I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37°C. After extensive washing, cells are released *via* trypsinization.

The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson ImmunoResearch Laboratories, Inc., West Grove, PA. The antibody is radioiodinated using the standard chloramine-T method. The antibody will bind to the Fc portion of any polypeptide/Fc protein that has bound to the cells. In all assays, non-specific binding of ¹²⁵I-antibody is assayed in the absence of the Fc fusion protein, as well as in the presence of the Fc fusion protein and a 200-fold molar excess of unlabeled mouse anti-human IgG antibody.

Cell-bound ¹²⁵I-antibody is quantified on a Packard Autogamma counter. Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) are generated on RS/1 (BBN Software, Boston, MA) run on a Microvax computer.

Another type of suitable binding assay is a competitive binding assay. To illustrate, biological activity of a variant may be determined by assaying for the variant's ability to compete

with the native protein for binding to IL-17D counterstructures or cells expressing a IL-17D counterstructure.

Competitive binding assays can be performed by conventional methodology. Reagents that may be employed in competitive binding assays include radiolabeled IL-17D and intact cells expressing IL-17D counterstructures (endogenous or recombinant) on the cell surface. For example, a radiolabeled soluble IL-17D fragment can be used to compete with a soluble IL-17D variant for binding to cell surface (binding partner). Instead of intact cells, one could substitute a soluble IL-17D counterstructure/Fc fusion protein bound to a solid phase through the interaction of Protein A or Protein G (on the solid phase) with the Fc moiety. Chromatography columns that contain Protein A and Protein G include those available from Pharmacia Biotech, Inc., Piscataway, NJ.

Another type of competitive binding assay utilizes radiolabeled soluble IL-17D counterstructure such as a soluble IL-17D counterstructure/Fc fusion protein, and intact cells expressing IL-17D. Qualitative results can be obtained by competitive autoradiographic plate binding assays, while Scatchard plots (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) may be utilized to generate quantitative results.

USE OF IL-17D NUCLEIC ACID OR OLIGONUCLEOTIDES

In addition to being used to express polypeptides as described above, the nucleic acids of the invention, including DNA, and oligonucleotides thereof can be used:

- as probes to identify nucleic acid encoding proteins having IL-17D activity;
- as single-stranded sense or antisense oligonucleotides, to inhibit expression of polypeptide encoded by the IL-17D gene;
- to help detect defective genes in an individual; and
- for gene therapy.

Probes

Among the uses of nucleic acids of the invention is the use of fragments as probes or primers. Such fragments generally comprise at least about 17 contiguous nucleotides of a DNA sequence. In other embodiments, a DNA fragment comprises at least 30, or at least 60, contiguous nucleotides of a DNA sequence.

Because homologs of SEQ ID NO:1 and SEQ ID NO:3 from other mammalian species are contemplated herein, probes based on the DNA sequence of SEQ ID NO:1 or SEQ ID NO:3 may be used to screen cDNA libraries derived from other mammalian species, using conventional cross-species hybridization techniques.

Using knowledge of the genetic code in combination with the amino acid sequences set forth above, sets of degenerate oligonucleotides can be prepared. Such oligonucleotides are useful as primers, e.g., in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified.

Sense-Antisense

Other useful fragments of the nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of DNA (SEQ ID NO:1 or SEQ ID NO:3). Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (*Cancer Res.* 48:2659, 1988) and van der Krol et al. (*BioTechniques* 6:958, 1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block or inhibit protein expression by one of several means, including enhanced degradation of the mRNA by RNaseH, inhibition of splicing, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating

agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, lipofection, CaPO₄-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus.

Sense or antisense oligonucleotides are preferably introduced into a cell containing the target nucleic acid sequence by insertion of the sense or antisense oligonucleotide into a suitable retroviral vector, then contacting the cell with the retrovirus vector containing the inserted sequence, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see PCT Application US 90/02656).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase. to a monoclonal antibody targeted to a specific cell type.

USE OF IL-17D POLYPEPTIDES AND FRAGMENTED POLYPEPTIDES

Uses include, but are not limited to, the following:

- Purifying proteins and measuring activity thereof
- Delivery Agents
- Therapeutic Agents
- Rational Drug Design
- Research Reagents

- Molecular weight and Isoelectric focusing markers
- Controls for peptide fragmentation
- Identification of unknown proteins
- Preparation of Antibodies

5

Purification Reagents

Each of the polypeptides of the invention finds use as a protein purification reagent. The polypeptides may be attached to a solid support material and used to purify IL-17D counter-structure molecules by affinity chromatography. In particular embodiments, a polypeptide (in any form described herein that is capable of binding IL-17D counter-structure molecules) is attached to a solid support by conventional procedures. As one example, chromatography columns containing functional groups that will react with functional groups on amino acid side chains of proteins are available (Pharmacia Biotech, Inc., Piscataway, NJ). In an alternative, a polypeptide/Fc protein (as discussed above) is attached to Protein A- or Protein G-containing chromatography columns through interaction with the Fc moiety.

The polypeptide also finds use in purifying or identifying cells that express IL-17D counter-structure molecules on the cell surface. Polypeptides are bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with the polypeptides and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing IL-17D counter-structure molecule-expressing cells are contacted with the solid phase having the polypeptides thereon. Cells expressing IL-17D counter-structure molecules on the cell surface bind to the fixed polypeptides, and unbound cells then are washed away.

Alternatively, the polypeptides can be conjugated to a detectable moiety, then incubated with cells to be tested for IL-17D counter-structure molecules expression. After incubation, unbound labeled matter is removed and the presence or absence of the detectable moiety on the cells is determined.

In a further alternative, mixtures of cells suspected of containing cells expressing IL-17D counter-structure molecules are incubated with biotinylated polypeptides. Incubation periods are typically at least one hour in duration to ensure sufficient binding. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides binding of the desired cells to the beads. Procedures for using avidin-coated

beads are known (see Berenson, et al. *J. Cell. Biochem.*, 10D:239, 1986). Washing to remove unbound material, and the release of the bound cells, are performed using conventional methods.

Measuring Activity

Polypeptides also find use in measuring the biological activity of IL-17D counter-structure molecules in terms of their binding affinity. The polypeptides thus may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of protein under different conditions. For example, the polypeptides may be employed in a binding affinity study to measure the biological activity of a IL-17D counter-structure molecule that has been stored at different temperatures, or produced in different cell types. The proteins also may be used to determine whether biological activity is retained after modification of a IL-17D counter-structure molecule (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified IL-17D counter-structure molecule is compared to that of an unmodified IL-17D counter-structure molecules to detect any adverse impact of the modifications on biological activity of IL-17D counter-structure molecules. The biological activity of a IL-17D counter-structure molecule thus can be ascertained before it is used in a research study, for example.

Delivery Agents

The polypeptides can be used to deliver diagnostic or therapeutic agents to such cells or cell types found to express IL-17D counterstructure molecules on the cell surface in *in vitro* or *in vivo* procedures. Therefore, IL-17D polypeptide can be attached to a toxin to bind to cells that express IL-17D counterstructure molecules on the cell surface and specifically kill these cells. The methodology can be similar to the successful use of an anti-CD72 immunotoxin to treat therapy-refractory B-lineage acute lymphoblastic leukemia in SCID mice (Meyers et al., *Leuk. and Lymph.* 18:119-122).

Detectable (diagnostic) and therapeutic agents that may be attached to a polypeptide include, but are not limited to, toxins, other cytotoxic agents, drugs, radionuclides, chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as trichothecenes, and derivatives and fragments (e.g., single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, ^{123}I , ^{131}I , $^{99\text{m}}\text{Tc}$,

¹¹¹In, and ⁷⁶Br. Examples of radionuclides suitable for therapeutic use are ¹³¹I, ²¹¹At, ⁷⁷Br, ¹⁸⁶Re, ¹⁸⁸Re, ²¹²Pb, ²¹²Bi, ¹⁰⁹Pd, ⁶⁴Cu, and ⁶⁷Cu.

Such agents may be attached to the polypeptide by any suitable conventional procedure. The polypeptide comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to polypeptides by using a suitable bifunctional chelating agent, for example.

Conjugates comprising polypeptides and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Therapeutic Agents

Polypeptides of the invention may be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of the polypeptides. These polypeptides may be administered to a mammal afflicted with such a disorder.

The polypeptides may also be employed in inhibiting a biological activity of IL-17D counterstructures, in *in vitro* or *in vivo* procedures. For example, a purified polypeptide may be used to inhibit binding of IL-17D polypeptides to endogenous cell surface IL-17D counterstructures. Biological effects that result from the binding of IL-17D to endogenous receptors thus are inhibited.

IL-17D may be administered to a mammal to treat a IL-17D counterstructure-mediated disorder. Such IL-17D counterstructure-mediated disorders include conditions caused (directly or indirectly) or exacerbated by IL-17D counterstructures.

Compositions of the present invention may contain a polypeptide in any form described herein, such as native proteins, variants, derivatives, oligomers, and biologically active fragments. In particular embodiments, the composition comprises a soluble polypeptide or an oligomer comprising soluble IL-17D polypeptides.

Compositions comprising an effective amount of a polypeptide of the present invention, in combination with other components such as a physiologically acceptable diluent, carrier, or

excipient, are provided herein. The polypeptides can be formulated according to known methods used to prepare pharmaceutically useful compositions. They can be combined in admixture, either as the sole active material or with other known active materials suitable for a given indication, with pharmaceutically acceptable diluents (e.g., saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (e.g., thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences*, 16th ed. 1980, Mack Publishing Company, Easton, PA.

In addition, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance, and are thus chosen according to the intended application.

The compositions of the invention can be administered in any suitable manner, e.g., topically, parenterally, or by inhalation. The term "parenteral" includes injection, e.g., by subcutaneous, intravenous, or intramuscular routes, also including localized administration, e.g., at a site of disease or injury. Sustained release from implants is also contemplated. One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature of the disorder to be treated, the patient's body weight, age, and general condition, and the route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices.

Compositions comprising nucleic acids in physiologically acceptable formulations are also contemplated. DNA may be formulated for injection, for example.

Rational Drug Design

In addition, IL-17D polypeptides can also be used for structure-based design of IL-17D inhibitors. Such structure-based design is also known as "rational drug design." The IL-17D polypeptides can be three dimensionally analyzed by, for example, X-ray crystallography, nuclear magnetic resonance, or homology modeling, all of which are well known methods. The use of IL-17D structural information in molecular modeling software systems to assist in inhibitor design and inhibitor-IL-17D interaction is also encompassed by the invention. Such

computer-assisted modeling and drug design may utilize information such as chemical conformational analysis, electrostatic potential of the molecules, protein folding, etc. For example, most of the design of class-specific inhibitors of metalloproteases has focused on attempts to chelate or bind the catalytic zinc atom. Synthetic inhibitors are usually designed to contain a negatively charged moiety to which is attached a series of other groups designed to fit the specificity pockets of the particular protease. A particular method of the invention comprises analyzing the three dimensional structure of IL-17D for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described above.

Research reagents

Another use of the polypeptide of the present invention is as a research tool for studying the biological effects that result from inhibiting IL-17D /IL-17D counter-structure interactions on different cell types. Polypeptides also may be employed in *in vitro* assays for detecting IL-17D counter-structure molecules or IL-17D polypeptides or the interactions thereof.

IL-17D may also be used as a reagent to identify (a) the proteins to which it binds, and which are involved in IL-17D signaling, and (b) other proteins with which it might interact which would be involved in signal transduction pathways. These other proteins would then be useful tools to search for other inhibitors of signaling. IL-17D could be used by coupling recombinant protein to an affinity matrix, or by using it as a bait in the yeast 2-hybrid system discussed below.

The interaction between IL-17D polypeptide and its counter-structure enables screening for small molecules that interfere with the IL-17D polypeptide/IL-17D counter-structure association and inhibit activity of IL-17D polypeptide or its counter-structure. For example, the yeast two-hybrid system developed at SUNY (described in U.S. Patent No. 5,283,173 to Fields et al.) may be used to screen for inhibitors of IL-17D as follows. IL-17D polypeptide and its counter-structure, or portions thereof responsible for their interaction, may be fused to the Gal 4 DNA binding domain and Gal 4 transcriptional activation domain, respectively, and introduced into a strain that depends on Gal 4 activity for growth on plates lacking histidine. Compounds that prevent growth may be screened in order to identify IL-17D inhibitors. Alternatively, the screen may be modified so that IL-17D polypeptide/IL-17D polypeptide counter-structure interaction inhibits growth, so that inhibition of the interaction allows growth to occur.

Another *in vitro* approach to screening for IL-17D inhibition would be to immobilize one of the components (either IL-17D polypeptide or its counter-structure) in wells of a microtiter plate, and to couple an easily detected indicator to the other component. An inhibitor of the interaction is identified by the absence of the detectable indicator from the well.

5 In addition, IL-17D polypeptides according to the invention are useful for the structure-based design of an IL-17D inhibitor. Such a design would comprise the steps of determining the three-dimensional structure of the IL-17D polypeptide, analyzing the three-dimensional structure for the likely binding sites of substrates, synthesizing a molecule that incorporates a predictive reactive site, and determining the inhibiting activity of the molecule.

10 IL-17D DNA, IL-17D polypeptides, and antibodies against IL-17D polypeptides can be used as reagents in a variety of research protocols. A sample of such research protocols are given in Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, (1989). For example, these reagents can serve as markers for cell specific or tissue specific expression of RNA or proteins. Similarly, these reagents can be used
15 to investigate constitutive and transient expression of IL-17D RNA or polypeptides. IL-17D DNA can be used to determine the chromosomal location of IL-17D DNA and to map genes in relation to this chromosomal location. IL-17D DNA can also be used to examine genetic heterogeneity and heredity, through the use of techniques such as genetic fingerprinting, as well as to identify risks associated with genetic disorders. IL-17D DNA can be further used to
20 identify additional genes related to IL-17D DNA and to establish evolutionary trees based on the comparison of sequences. IL-17D DNA and polypeptides can be used to select for those genes or proteins that are homologous to IL-17D DNA or polypeptides through positive screening procedures, such as Southern blotting and immunoblotting, and through negative screening procedures, such as subtraction.

25 Molecular Weight, Isoelectric Point Markers

The polypeptides of the present invention can be subjected to fragmentation into smaller peptides by chemical and enzymatic means, and the peptide fragments so produced can be used in the analysis of other proteins or polypeptides. For example, such peptide fragments can be
30 used as peptide molecular weight markers, peptide isoelectric point markers, or in the analysis of the degree of peptide fragmentation. Thus, the invention also includes these polypeptides and peptide fragments, as well as kits to aid in the determination of the apparent molecular weight

and isoelectric point of an unknown protein and kits to assess the degree of fragmentation of an unknown protein.

Although all methods of fragmentation are encompassed by the invention, chemical fragmentation is a preferred embodiment, and includes the use of cyanogen bromide to cleave under neutral or acidic conditions such that specific cleavage occurs at methionine residues (E. Gross, *Methods in Enz.* 11:238-255, 1967). This can further include additional steps, such as a carboxymethylation step to convert cysteine residues to an unreactive species.

Enzymatic fragmentation is another preferred embodiment, and includes the use of a protease such as Asparaginylendo-peptidase, Arginylendo-peptidase, *Achromobacter* protease I, Trypsin, *Staphylococcus aureus* V8 protease, Endoproteinase Asp-N, or Endoproteinase Lys-C under conventional conditions to result in cleavage at specific amino acid residues.

Asparaginylendo-peptidase can cleave specifically on the carboxyl side of the asparagine residues present within the polypeptides of the invention. Arginylendo-peptidase can cleave specifically on the carboxyl side of the arginine residues present within these polypeptides.

Achromobacter protease I can cleave specifically on the carboxyl side of the lysine residues present within the polypeptides (Sakiyama and Nakat, U.S. Patent No. 5,248,599; T. Masaki et al., *Biochim. Biophys. Acta* 660:44-50, 1981; T. Masaki et al., *Biochim. Biophys. Acta* 660:51-55, 1981). Trypsin can cleave specifically on the carboxyl side of the arginine and lysine residues present within polypeptides of the invention. Enzymatic fragmentation may also occur with a protease that cleaves at multiple amino acid residues. For example, *Staphylococcus aureus* V8 protease can cleave specifically on the carboxyl side of the aspartic and glutamic acid residues present within polypeptides (D. W. Cleveland, *J. Biol. Chem.* 3:1102-1106, 1977). Endoproteinase Asp-N can cleave specifically on the amino side of the asparagine residues present within polypeptides. Endoproteinase Lys-C can cleave specifically on the carboxyl side of the lysine residues present within polypeptides of the invention. Other enzymatic and chemical treatments can likewise be used to specifically fragment these polypeptides into a unique set of specific peptides.

Of course, the peptides and fragments of the polypeptides of the invention can also be produced by conventional recombinant processes and synthetic processes well known in the art. With regard to recombinant processes, the polypeptides and peptide fragments encompassed by invention can have variable molecular weights, depending upon the host cell in which they are expressed. Glycosylation of polypeptides and peptide fragments of the invention in various cell

types can result in variations of the molecular weight of these pieces, depending upon the extent of modification. The size of these pieces can be most heterogeneous with fragments of polypeptide derived from the extracellular portion of the polypeptide. Consistent polypeptides and peptide fragments can be obtained by using polypeptides derived entirely from the transmembrane and cytoplasmic regions, pretreating with N-glycanase to remove glycosylation, or expressing the polypeptides in bacterial hosts.

The molecular weight of these polypeptides can also be varied by fusing additional peptide sequences to both the amino and carboxyl terminal ends of polypeptides of the invention. Fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention can be used to enhance expression of these polypeptides or aid in the purification of the protein. In addition, fusions of additional peptide sequences at the amino and carboxyl terminal ends of polypeptides of the invention will alter some, but usually not all, of the fragmented peptides of the polypeptides generated by enzymatic or chemical treatment. Of course, mutations can be introduced into polypeptides of the invention using routine and known techniques of molecular biology. For example, a mutation can be designed so as to eliminate a site of proteolytic cleavage by a specific enzyme or a site of cleavage by a specific chemically induced fragmentation procedure. The elimination of the site will alter the peptide fingerprint of polypeptides of the invention upon fragmentation with the specific enzyme or chemical procedure.

The polypeptides and the resultant fragmented peptides can be analyzed by methods including sedimentation, electrophoresis, chromatography, and mass spectrometry to determine their molecular weights. Because the unique amino acid sequence of each piece specifies a molecular weight, these pieces can thereafter serve as molecular weight markers using such analysis techniques to assist in the determination of the molecular weight of an unknown protein, polypeptides or fragments thereof. The molecular weight markers of the invention serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of proteins that have similar apparent molecular weights and, consequently, allow increased accuracy in the determination of apparent molecular weight of proteins.

When the invention relates to the use of IL-17D polypeptide fragments and fragmented peptide molecular weight markers, those markers are preferably at least 10 amino acids in size. More preferably, these fragmented peptide molecular weight markers are between 10 and 100 amino acids in size. Even more preferable are fragmented peptide molecular weight markers

between 10 and 50 amino acids in size and especially between 10 and 35 amino acids in size. Most preferable are fragmented peptide molecular weight markers between 10 and 20 amino acids in size.

Among the methods for determining molecular weight are sedimentation, gel electrophoresis, chromatography, and mass spectrometry. A particularly preferred embodiment is denaturing polyacrylamide gel electrophoresis (U. K. Laemmli, *Nature* 227:680-685, 1970). Conventionally, the method uses two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 6-20%. The ability to simultaneously resolve the marker and the sample under identical conditions allows for increased accuracy. It is understood, of course, that many different techniques can be used for the determination of the molecular weight of an unknown protein using polypeptides of the invention, and that this embodiment in no way limits the scope of the invention.

Each unglycosylated polypeptide or fragment thereof has a pI that is intrinsically determined by its unique amino acid sequence (which pI can be estimated by the skilled artisan using any of the computer programs designed to predict pI values currently available, calculated using any well-known amino acid pKa table, or measured empirically). Therefore these polypeptides and fragments thereof can serve as specific markers to assist in the determination of the isoelectric point of an unknown protein, polypeptide, or fragmented peptide using techniques such as isoelectric focusing. These polypeptide or fragmented peptide markers serve particularly well for the estimation of apparent isoelectric points of unknown proteins that have apparent isoelectric points close to that of the polypeptide or fragmented peptide markers of the invention.

The technique of isoelectric focusing can be further combined with other techniques such as gel electrophoresis to simultaneously separate a protein on the basis of molecular weight and charge. The ability to simultaneously resolve these polypeptide or fragmented peptide markers and the unknown protein under identical conditions allows for increased accuracy in the determination of the apparent isoelectric point of the unknown protein. This is of particular interest in techniques, such as two dimensional electrophoresis (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)), where the nature of the procedure dictates that any markers should be resolved simultaneously with the unknown protein. In addition, with such methods, these polypeptides and fragmented peptides thereof can assist in the determination of both the isoelectric point and molecular weight of an unknown protein or fragmented peptide.

Polypeptides and fragmented peptides can be visualized using two different methods that allow a discrimination between the unknown protein and the molecular weight markers. In one embodiment, the polypeptide and fragmented peptide molecular weight markers of the invention can be visualized using antibodies generated against these markers and conventional immunoblotting techniques. This detection is performed under conventional conditions that do not result in the detection of the unknown protein. It is understood that it may not be possible to generate antibodies against all polypeptide fragments of the invention, since small peptides may not contain immunogenic epitopes. It is further understood that not all antibodies will work in this assay; however, those antibodies which are able to bind polypeptides and fragments of the invention can be readily determined using conventional techniques.

The unknown protein is also visualized by using a conventional staining procedure. The molar excess of unknown protein to polypeptide or fragmented peptide molecular weight markers of the invention is such that the conventional staining procedure predominantly detects the unknown protein. The level of these polypeptide or fragmented peptide molecular weight markers is such as to allow little or no detection of these markers by the conventional staining method. The preferred molar excess of unknown protein to polypeptide molecular weight markers of the invention is between 2 and 100,000 fold. More preferably, the preferred molar excess of unknown protein to these polypeptide molecular weight markers is between 10 and 10,000 fold and especially between 100 and 1,000 fold.

It is understood of course that many techniques can be used for the determination and detection of molecular weight and isoelectric point of an unknown protein, polypeptides, and fragmented peptides thereof using these polypeptide molecular weight markers and peptide fragments thereof and that these embodiments in no way limit the scope of the invention.

In another embodiment, the analysis of the progressive fragmentation of the polypeptides of the invention into specific peptides (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977), such as by altering the time or temperature of the fragmentation reaction, can be used as a control for the extent of cleavage of an unknown protein. For example, cleavage of the same amount of polypeptide and unknown protein under identical conditions can allow for a direct comparison of the extent of fragmentation. Conditions that result in the complete fragmentation of the polypeptide can also result in complete fragmentation of the unknown protein.

Thus, the IL-17D polypeptides can be used as molecular weight markers to estimate the apparent molecular weight of a sample protein by gel electrophoresis. An isolated and purified

human IL-17D polypeptide has a molecular weight of approximately 20,423 Daltons, in the absence of glycosylation while isolated and purified mouse IL-17D polypeptide has a molecular weight of approximately 20,295 Daltons, in the absence of glycosylation. Therefore, the human IL-17D polypeptide molecular weight marker serves particularly well as a molecular weight marker for the estimation of the apparent molecular weight of sample proteins that have apparent molecular weights close to 20,423 Daltons. The use of this polypeptide molecular weight marker allows an increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 20,423 Daltons.

Another preferred embodiment of the invention is the use of IL-17D fragments generated by chemical fragmentation of IL-17D polypeptide, as molecular weight markers. Isolated and purified IL-17D polypeptide can be treated with cyanogen bromide under conventional conditions that result in fragmentation of the IL-17D polypeptide molecular weight marker by specific hydrolysis on the carboxyl side of the methionine residues within the IL-17D polypeptide (E. Gross, *Methods in Enz.* 11:238-255, 1967), as noted above. The unique set of IL-17D fragmented peptide molecular weight markers generated by treatment of human IL-17D polypeptide with cyanogen bromide comprises 6 fragmented peptides of at least 10 amino acids in size. The peptide encoded by amino acids 2-53 of SEQ ID NO:2 has a molecular weight of approximately 5,721 Daltons. The peptide encoded by amino acids 60-69 of SEQ ID NO:2 has a molecular weight of approximately 1,340 Daltons. The peptide encoded by amino acids 70-92 of SEQ ID NO:2 has a molecular weight of approximately 2,715 Daltons. The peptide encoded by amino acids 93-132 of SEQ ID NO:2 has a molecular weight of approximately 4,472 Daltons. The peptide encoded by amino acids 139-169 of SEQ ID NO:2 has a molecular weight of approximately 3,484 Daltons. The peptide encoded by amino acids 170-180 of SEQ ID NO:2 has a molecular weight of approximately 1,155 Daltons.

Therefore, cleavage of the IL-17D polypeptide by chemical treatment with cyanogen bromide generates a unique set of IL-17D fragments having molecular weights of approximately 5,721; 1,340; 2,715; 4,472; 3,484; and 1,155 Daltons. Therefore, the IL-17D fragmented peptide molecular weight markers serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of sample proteins that have apparent molecular weights close to 5,721; 1,340; 2,715; 4,472; 3,484; or 1,155 Daltons. Consequently, the use of these fragmented peptide molecular weight markers allows an increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 5,721;

1,340; 2,715; 4,472; 3,484; or 1,155 Daltons. Similarly, the set of fragmented peptide molecular weight markers generated by cyanogen bromide treatment of mouse IL-17D polypeptide have molecular weights of approximately 6,446; 1,268; 7,079; 3,539; and 1,155 Daltons.

5 The extent of fragmentation of the IL-17D polypeptide is further used as a control to determine the conditions expected for complete fragmentation of the sample protein. It is understood of course that many chemicals could be used to fragment IL-17D polypeptides and that this embodiment in no way limits the scope of the invention.

10 In another embodiment, unique sets of IL-17D fragmented peptide molecular weight markers can be generated from IL-17D polypeptide using enzymes that cleave the polypeptide at specific amino acid residues. Due to the unique nature of the amino acid sequence of the IL-17D polypeptide, cleavage at different amino acid residues will result in the generation of different sets of fragmented peptide molecular weight markers.

15 An isolated and purified IL-17D polypeptide can be treated, as noted above, with *Achromobacter* protease I under conventional conditions that result in fragmentation of the IL-17D polypeptide by specific hydrolysis on the carboxyl side of the lysine residues within the IL-17D polypeptide (T. Masaki et al., *Biochim. Biophys. Acta* 660:44-50, 1981; T. Masaki et al., *Biochim. Biophys. Acta* 660:51-55, 1981).

20 The unique set of IL-17D fragments generated by treatment comprises 5 fragmented peptides of at least 10 amino acids in size. The generation of 5 fragmented peptides with this enzyme treatment of the IL-17D polypeptide, compared to 6 fragmented peptides with cyanogen bromide treatment of the IL-17D polypeptide, clearly illustrates that both the size and number of the fragmented peptide molecular weight markers will vary depending upon the fragmentation treatment utilized to fragment the IL-17D polypeptide.

25 The peptide encoded by amino acids 1-26 of SEQ ID NO:2 has a molecular weight of approximately 2,992 Daltons. The peptide encoded by amino acids 31-54 of SEQ ID NO:2 has a molecular weight of approximately 2,506 Daltons. The peptide encoded by amino acids 55-83 of SEQ ID NO:2 has a molecular weight of approximately 3,539 Daltons. The peptide encoded by amino acids 84-95 of SEQ ID NO:2 has a molecular weight of approximately 1,463 Daltons. The peptide encoded by amino acids 96-180 of SEQ ID NO:2 has a molecular weight of approximately 9,493 Daltons. Thus, these IL-17D fragmented peptide molecular weight markers have molecular weights of approximately 2,992; 2,506; 3,539; 1,463; and 9,493 Daltons. In contrast, the fragmented peptide molecular weight markers generated by *Achromobacter* protease

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I treatment of mouse IL-17D polypeptide (SEQ ID NO:4) have molecular weights of approximately 3,003; 2,504; 3,167; 1,445; and 9,520 Daltons.

The extent of fragmentation of the IL-17D polypeptide is further used as a control to determine the conditions expected for complete fragmentation of the sample protein. It is understood of course that many enzymes could be used to fragment IL-17D polypeptides and that this embodiment in no way limits the scope of the invention.

Finally, as to the kits that are encompassed by the invention, the constituents of such kits can be varied, but typically contain the polypeptide and fragmented peptide molecular weight markers. Also, such kits can contain the polypeptides wherein a site necessary for fragmentation has been removed. Furthermore, the kits can contain reagents for the specific cleavage of the polypeptide and the unknown protein by chemical or enzymatic cleavage. Kits can further contain antibodies directed against polypeptides or fragments thereof of the invention.

Identification of Unknown Proteins

As set forth above, a polypeptide or peptide fingerprint can be entered into or compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., Proc. Natl. Acad. Sci. USA 90:5011-5015, 1993; D. Fenyo et al., Electrophoresis 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heidelberg.de/deSearch/FR_PeptideSearchForm.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare observed molecular weights to predicted peptide molecular weights derived from sequence databases to assist in determining the identity of the unknown protein.

In addition, a polypeptide or peptide digest can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., J. Am. Soc. Mass Spec. 5:976-989 (1994); M. Mann and M. Wilm, Anal. Chem. 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, Rapid Comm. Mass Spec. 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97

(Internet site: www.lsb.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search and ProFound programs described above.

Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using mass spectrometry.

Antibodies

Antibodies that are immunoreactive with the polypeptides of the invention are provided herein. Such antibodies specifically bind to the polypeptides *via* the antigen-binding sites of the antibody (as opposed to non-specific binding). Thus, the polypeptides, fragments, variants, fusion proteins, etc., as set forth above may be employed as immunogens in producing antibodies immunoreactive therewith. More specifically, the polypeptides, fragment, variants, fusion proteins, etc. contain antigenic determinants or epitopes that elicit the formation of antibodies.

These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acid sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 3:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the protein and steric hinderances, the number of antibodies that actually bind to the epitopes is less than the number of available epitopes (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 2:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes may be identified by any of the methods known in the art.

Thus, one aspect of the present invention relates to the antigenic epitopes of the polypeptides of the invention. Such epitopes are useful for raising antibodies, in particular monoclonal antibodies, as described in more detail below. Additionally, epitopes from the polypeptides of the invention can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques well known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

As to the antibodies that can be elicited by the epitopes of the polypeptides of the invention, whether the epitopes have been isolated or remain part of the polypeptides, both polyclonal and monoclonal antibodies may be prepared by conventional techniques as discussed below.

5 Thus, in this aspect of the invention, IL-17D and peptides based on the amino acid sequence of IL-17D, can be utilized to prepare antibodies that specifically bind to IL-17D. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, fragments thereof, such as F(ab')₂ and Fab fragments, as well as any recombinantly produced binding partners. Antibodies are defined to be specifically binding if they bind IL-17D polypeptide with
10 a K_a of greater than or equal to about 10^7 M^{-1} . Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, using procedures that are well
15 known in the art. In general, purified IL-17D or a peptide based on the amino acid sequence of IL-17D polypeptide that is appropriately conjugated is administered to the host animal typically through parenteral injection. The immunogenicity of IL-17D polypeptide can be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity
20 to IL-17D polypeptide. Examples of various assays useful for such determination include those described in *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures, such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radio-immunoprecipitation, enzyme-linked immunosorbent assays (ELISA), dot blot assays, and sandwich assays. See U.S. Patent Nos. 4,376,110 and 4,486,530.

25 Monoclonal antibodies can be readily prepared using well known procedures. See, for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980. Briefly, the host animals, such as mice, are injected intraperitoneally at least once and preferably at least twice at about 3 week
30 intervals with isolated and purified IL-17D or conjugated IL-17D peptide, optionally in the presence of adjuvant. Mouse sera are then assayed by conventional dot blot technique or antibody capture (ABC) to determine which animal is best to fuse. Approximately two to three

weeks later, the mice are given an intravenous boost of IL-17D or conjugated IL-17D peptide. Mice are later sacrificed and spleen cells fused with commercially available myeloma cells, such as Ag8.653 (ATCC), following established protocols. Briefly, the myeloma cells are washed several times in media and fused to mouse spleen cells at a ratio of about three spleen cells to one myeloma cell. The fusing agent can be any suitable agent used in the art, for example, polyethylene glycol (PEG). Fusion is plated out into plates containing media that allows for the selective growth of the fused cells. The fused cells can then be allowed to grow for approximately eight days. Supernatants from resultant hybridomas are collected and added to a plate that is first coated with goat anti-mouse Ig. Following washes, a label, such as ¹²⁵I-IL-17D, is added to each well followed by incubation. Positive wells can be subsequently detected by autoradiography. Positive clones can be grown in bulk culture and supernatants are subsequently purified over a Protein A column (Pharmacia).

The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3:1-9 (1990), which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7:394 (1989).

The monoclonal antibodies of the present invention include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature* 332:323, 1988), Liu et al. (*PNAS* 84:3439, 1987), Larrick et al. (*Bio/Technology* 7:934, 1989), and Winter and Harris (*TIPS* 14:139, May, 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, US Patent Nos. 5,569,825 and

5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein.

Uses Thereof

5 The antibodies of the invention can be used in assays to detect the presence of the polypeptides or fragments of the invention, either *in vitro* or *in vivo*. The antibodies also may be employed in purifying polypeptides or fragments of the invention by immunoaffinity chromatography.

10 Those antibodies that additionally can block binding of the polypeptides of the invention to IL-17D counter-structure molecules may be used to inhibit a biological activity that results from such binding. Such blocking antibodies may be identified using any suitable assay procedure, such as by testing antibodies for the ability to inhibit binding of IL-17D polypeptides to certain cells expressing IL-17D counter-structure molecules. Alternatively, blocking antibodies may be identified in assays for the ability to inhibit a biological effect that results
15 from binding of IL-17D counter-structure molecules to target cells. Antibodies may be assayed for the ability to inhibit IL-17D counter-structure molecules-mediated lysis of cells, for example.

20 Such an antibody may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit a biological activity mediated by the entity that generated the antibody. Disorders caused or exacerbated (directly or indirectly) by the interaction of IL-17D counter-structure molecules with cell surface (binding partner) receptor thus may be treated. A therapeutic method involves
25 *in vivo* administration of a blocking antibody to a mammal in an amount effective in inhibiting a IL-17D counter-structure molecule-mediated biological activity. Monoclonal antibodies are generally preferred for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is employed.

30 Compositions comprising an antibody that is directed against IL-17D polypeptides, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described above for compositions containing IL-17D polypeptides.

35 Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to the antibody. Examples of such agents are presented above. The conjugates find use in *in vitro* or *in vivo* procedures.

 The references cited herein are incorporated by reference herein in their entirety.

The embodiments within the specification and the following examples provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan recognizes many other embodiments are encompassed by the claimed invention.

EXAMPLE 1**Recombinant Expression of IL-17D****Fc Fusion Proteins**

Both human and mouse 17D/mutFc DNA constructs were made to express human and mouse 17D-immunoglobulin Fc fusion proteins, referred to as human or mouse 17D/mutFc, respectively.

DNA encoding human or mouse 17D/mutFc comprises sequences encoding:

a leader (or signal) peptide

either the human IL-7 leader or the murine IL-7 leader,

an octapeptide referred to as Flag, as described above,

a suitable Fc region of an immunoglobulin mutated to minimize binding to Fc receptor (Baum et al.),

a flexible linker sequence, and

a portion of the 17D gene, as follows:

human 17D from amino acid 21 (computer-predicted signal cleavage site is after amino acid 20, with a score of 8.6) to amino acid 180

mouse 17D from amino acid 21 (computer-predicted signal cleavage site is after amino acid 20 with a score of 7.7) to amino acid 180, both plus a stop codon.

An expression vector containing a leader sequence, Flag, mutated hu IgG Fc and flexible linker was prepared using conventional techniques of enzyme cutting and ligation of fragments. The resulting vector was then restricted with SpeI and NotI. The human or mouse 17D was inserted 5' to 3' after the flexible linker in a two-way ligation described below.

PCR was employed using 5' (upstream) and 3' (downstream) oligonucleotide primers to amplify the DNA sequences encoding human or mouse 17D from EST DNA to form a PCR fragment. For the human sequence, human 17D from EST DNA (Research Genetics Imageclone ID #375689, DBEST #647854, entry created 8/23/96) was used, and for the mouse sequence, EST DNA (Research Genetics Imageclone ID#405946, DB3ST#675514, entry created 9/12/96) was used.

Oligonucleotide primers were designed based on the sequence of the ESTs. The upstream oligonucleotide primer introduced a SpeI site upstream of amino acid 32 of the human 17D peptide. A downstream oligonucleotide primer introduced a NotI site just downstream of the termination codon after amino acid 180.

The primers used were:

upstream SpeI primer

5'-TGTCAGTCTAGTCAGCCCAGCAGCCCCAAAAGCAAG-3'

downstream NotI primer

5'-GACAGCGGCCGCTCAGAAGATGCAGGTGCAGCCCACA-3'

For mouse IL-17D, the upstream oligonucleotide primer introduced a SpeI site upstream of amino acid 21 of the mouse 17D peptide. A downstream oligonucleotide primer introduced a NotI site just downstream of the termination codon after amino acid 180.

upstream SpeI primer

5'-TGTCAGTCTAGTCACCCCCGGAACACCAAAGGCAA-3'

downstream NotI primer

5'-GACAGCGGCCGCTCAGAAGATGCAGGTGCAACCCAC-3'

The PCR fragment was then ligated into an expression vector (pDC409, a mammalian expression vector described in Giri et al. (*EMBO J.* 13:2822, 1994) containing a leader sequence, a flag sequence, mutated human IgG Fc and a flexible linker region in a two-way ligation. The resultant DNA construct (human or mouse 17D/mutFc) was transfected into the monkey kidney cell lines CV-1/EBNA.

The fusion proteins were expressed and then purified with the BioCad system.

PolyHIS fusion proteins

Both human and mouse IL-17D/polyHIS DNA constructs were made to express human and mouse 17D-immunoglobulin poly histidine-tagged fusion proteins.

DNA encoding human or mouse 17D/polyHIS comprises sequences encoding:

a CMV leader (or signal) peptide

from the 29080 ORF of CMV, having the sequence:

5'- ATGGCTCGGAGGCTATGGATCTTGAGCTTACTA
GCCGTGACCTTGACGGTGGCTTTGGCGGCACCTTCTCAG
-3'

a string of six histidine residues, having the sequence:

5'- CATCACCATCACCATCAC -3'

an octapeptide referred to as Flag, as described above,

a Factor X cleavage site, having the sequence:

5'- ATAGAAGGAGGA -3'

a glycine and two serine residues for flexibility, and
a portion of the 17D gene, as follows:

human 17D from amino acid 21 (computer-predicted signal cleavage site
is after amino acid 20, with a score of 8.6) to amino acid 180
mouse 17D from amino acid 21 (computer-predicted signal cleavage site
is after amino acid 20 with a score of 7.7) to amino acid 180, both plus a
stop codon.

An expression vector containing a leader sequence, polyHIS sequence, Flag, and a factor
Xa cleavage site was prepared using conventional techniques of enzyme cutting and ligation of
fragments. The resulting vector was then restricted with Bgl2 and Not1. The human or mouse
17D was inserted 5' to 3' after the flexible linker in a two-way ligation described below.

PCR was employed using 5' (upstream) and 3' (downstream) oligonucleotide primers to
amplify the DNA sequences encoding human or mouse 17D from EST DNA to form a PCR
fragment. For the human sequence, human 17D from EST DNA (Research Genetics Image
clone ID #375689, DBEST #647854, entry created 8/23/96) was used, and for the mouse
sequence, EST DNA (Research Genetics Image clone ID#405946, DB3ST#675514, entry created
9/12/96) was used.

Oligonucleotide primers were designed based on the sequence of the ESTs. The
upstream oligonucleotide primer introduced a Bgl2 site, one glycine, and two serine residues
upstream of amino acid 21 of the human 17D peptide. A downstream oligonucleotide primer
introduced a Not1 site just downstream of the termination codon after amino acid 180.

The primers used in the human construct were:

upstream Bgl2 primer

5'- CGATACGTAGATCTAGGTTCAAGTCAGCCCAGCAGCCCCAAA -3'

downstream Not1 primer

5'- GCTAGGACTAGCGGCCGCTCAGAAGATGCA -3'

For mouse IL-17D, the primers were

upstream Bgl2 primer

5'- CGATACGTAGATCTAGGTTCAAGTCACCCCCGGAACACCAAAA -3'

downstream Not1 primer

5'- GCTAGGACTAGCGGCCGCTCAGAAGATGCA -3'

The PCR fragment was then ligated into an expression vector (pDC409, a mammalian expression vector described in Giri et al. (*EMBO J.* 13:2822, 1994) containing a CMV leader sequence, a polyHIS sequence, a flag sequence, a factor Xa cleavage site in a two way ligation. The resultant DNA construct (human or mouse 17D/polyHIS) was transfected into the monkey kidney cell lines CV-1/EBNA.

The fusion proteins were expressed and then purified with the BioCad system.

EXAMPLE 2

Preparation of Antibodies Against Human IL-17D

Monoclonal antibodies (designated M491, M492, M493, and M494) were generated against human IL-17D. Balb/c mice were immunized with 10 μ g of Human IL-17D-Flag-polyHis in Titermax adjuvant (CytRx Corp., Norcross, GA). The animals were boosted 2 weeks later with 10 μ g of the same protein in Freund's Complete Adjuvant (Sigma). Twelve weeks after second immunization, one mouse was boosted intravenously with 10 μ g of the same protein in saline. Four days later, the mouse was sacrificed and spleen and lymph nodes were fused with NS1 myelomas with 50% PEG/DMSO solution. The fused cells were placed into 96 well plates (Costar) with HAT (Sigma) selective media. Hybridoma supernatants were screened for antibody production by ELISA with human IL-17D-Flag-Fc and by RIP with human IL-17D-Flag-polyHis

EXAMPLE 3

Screening Cell Lines for Binding to IL-17D Polypeptide

The IL-17D/Fc fusion protein prepared as described in Example 1 was used to screen cell lines for binding using quantitative binding studies according to standard flow cytometry methodologies. For each cell line screened, the procedure involved incubating approximately 100,000 of the cells blocked with 2% FCS (fetal calf serum), 5% normal goat serum and 5% rabbit serum in PBS for 1 hour. The blocked cells were then incubated with 5 μ g/mL of IL-17D/Fc fusion protein in 2% FCS, 5% goat serum and 5% rabbit serum in PBS. Following the incubation, the sample was washed 2 times with FACS buffer (2% FCS in PBS) and then treated with mouse anti human Fc/biotin (purchased from Jackson Research) and SAPE (streptavidin-

phycoerythrin purchased from Molecular Probes). This treatment causes the antihuman FC/biotin to bind to any bound IL-17D/Fc and the SAPE to bind to the anti-human Fc/biotin resulting in a fluorescent identifying label on IL-17D/Fc which is bound to cells. The cells were analyzed for any bound protein using fluorescent detection flow cytometry.

5 The results indicated that IL-17D binds specifically to suspension cell lines EL40.5, TF-1, S49.1, and K299 and showed significant binding to the B220+ population of mouse splenocytes (predominantly B220+/IgM+/IgD+). In addition, binding believed to be non-specific was found to adherent cell lines HFF, WI26, HT080T, CV-1, COS, 293ebna, TMOF, AND SK-Hep.

EXAMPLE 4

IL-17D RNA Expression by RT-PCR

To determine the presence of specific RNA message, PCR was performed on first strand cDNA generated from RNA isolated from cells or tissues. RNA was extracted and purified according to established procedures from cell lines and tissues of interest. First strand cDNA was generated from total polyA+ RNA using a First Strand cDNA Synthesis Kit from Amersham Pharmacia Biotech, Piscataway, N.J.

15 PCR was employed using two nested sets of 5' (sense) and 3' (anti-sense) oligonucleotide primers specific to the human and mouse IL-17D sequences to amplify identical regions from the cDNA.

20 These nested PCR primers have the following nucleotide sequences:

human sense:

5'- ATGGACTGGCCTCACAACCT-3'

nested human sense:

25 5'- GCCCCTGGCCCTCACCAGGT -3'

human anti-sense:

5'- TCAGAAGATGCAGGTGCAGC -3'

nested human anti-sense:

5'- GGAACCTGGCTGAACACCGG -3'

30 mouse sense:

5'- ATGGACTGGCCGCACAGCCT -3'

nested mouse sense:

5'- ATCAGGTGCCGCTGGACCTG -3'

mouse anti-sense:

5'- TCAGAAGATGCAGGTGCAAC -3'

nested mouse anti-sense:

5'- GGCTGAACACTGGCACGCTC -3'

Two rounds of PCR were performed. In the first round the PCR reaction mixture included 1 ul of the target cDNA, 50 pmoles each of sense and anti-sense primers (as above) for human or mouse 17D (dependent on the species of the cell line or tissue type cDNA), 1X Amplitaq buffer (Perkin-Elmer Cetus, Roche Molecular Systems Inc., Branchburg, NJ), 1.25 mM of dATP, dGTP, dTTP, dCTP; 0.1 ul of a 16:1 mix Klen-Taq:Vent polymerases (Ab Peptides Inc., St. Louis, MO and New England Biolabs, Beverly, MA, respectively) in a 25 ul final reaction volume. The PCR reaction cycles included one cycle at 94°C for 5 minutes; thirty cycles of: 94°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute; and 1 cycle at 72°C for 5 minutes using a Robocycler 96 (Stratagene, La Jolla, CA). The second round of PCR was performed as above using the nested primer pairs and 1 ul of a 1:50 dilution of the first round product. 5 ul of the second round product was run on a 1% agarose gel and transferred via Southern blot (Manniat's) to a nitrocellulose membrane. Membranes were hybridized following established procedures with a 32P-end kinased internal oligonucleotide probe specific for the human or mouse 17D (see below). Hybridized membranes were washed following established procedures and exposed to autoradiographic film. Cell lines or tissues with bands of the correct size (329 bp for the human 17 or 309 bp for the mouse 17D) were identified as positive for 17D.

human oligonucleotide probe

5'- GTCAACTTGCAGCTGTGG -3'

mouse oligonucleotide probe

5'- CCAGCCAAGAAGAAATGTGAAG -3'

RNA expression was detected in A549 and THP-1 cells, but not in primary peripheral blood T cells, primary NK cells, primary neutrophils, primary monocytes, foreskin fibroblasts, Jurkat cells, and JM-1 cells.

The specification is most thoroughly understood in light of the teachings of the references cited within the specification which are hereby incorporated by reference. The embodiments within the specification provide an illustration of embodiments of the invention and should not

be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:
 - (a) the DNA sequence of SEQ ID NO:1 or SEQ ID NO:3;
 - 5 (b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence of SEQ ID NO:2 or SEQ ID NO:4;
 - (c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing
 - 10 conditions of 60°C, 0.5XSSC, 0.1% SDS;
 - (d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:1 or SEQ ID NO:3;
 - (e) an isolated nucleic acid molecule degenerate from SEQ ID NO:1 or SEQ ID NO:3 as a result of the genetic code; and
 - 15 (f) an isolated nucleic acid molecule selected from the group consisting of human IL-17D DNA, mouse IL-17D DNA, an allelic variant of human IL-17D DNA, an allelic variant of mouse IL-17D DNA, and a species homolog of IL-17D DNA.
2. A recombinant vector that directs the expression of the nucleic acid molecule of
- 20 claim 1.
3. An isolated polypeptide encoded by the nucleic acid molecule of claim 1.
4. An isolated polypeptide according to claim 3 having a molecular weight of
- 25 approximately 20 kD as determined by SDS-PAGE.
5. An isolated polypeptide according to claim 3 in non-glycosylated form.
6. Isolated antibodies that bind to a polypeptide of claim 3.
- 30 7. Isolated antibodies according to claim 6, wherein the antibodies are monoclonal antibodies.

8. A host cell transfected or transduced with the vector of claim 2.

9. A method for the production of IL-17D polypeptide comprising culturing a host cell of claim 2 under conditions promoting expression, and recovering the polypeptide from the culture medium.

10. The method of claim 9, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

11. A method for the determination of the molecular weight of a sample protein comprising comparing molecular weight of a sample protein with the molecular weight of a polypeptide of claim 3;

wherein the comparison of molecular weights comprises application of the sample protein and polypeptide to an acrylamide gel, resolution of the sample protein and polypeptide using an electrical current, and application to the gel of a detection reagent, which stains the sample protein and polypeptide.

12. A kit for the determination of the molecular weights of peptide fragments of a sample protein comprising the following:

a vessel;

a polypeptide of claim 3;

at least one enzyme selected from the group consisting of

Asparaginylendopeptidase, Arginylendopeptidase, *Achromobacter* protease I, Trypsin, *Staphylococcus aureus* V8 protease, Endoproteinase Asp-N, and Endoproteinase Lys-C;

a mutated polypeptide from said polypeptide by *in vitro* mutagenesis, wherein a site of enzymatic cleavage by the selected enzyme has been removed; and

fragmented peptides derived from said peptide by enzymatic cleavage with the selected enzyme;

wherein said polypeptide and said sample protein are contacted with the selected protease; and wherein the protein, polypeptides, and fragmented peptides are visualized by application of the protein, polypeptides, and fragmented peptides to an acrylamide gel, resolution

SEQUENCE LISTING

<110> Spriggs, Melanie

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Pro Val Asp Leu Pro Glu Ala Arg Cys Leu Cys Leu Gly Cys Val Asn

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Thr Cys Ile Phe
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/00513

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/19 C07K14/52 C07K16/24 C12N5/10 C12N1/21
C12N1/19 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	" The WashU merck EST project" EMBL DATABASE ENTRY HSA33733, ACCESSION NUMBER AA033733, 26 August 1996, XP002073848 see the whole document ---	1,2
X	"The WashU-HHMI mouse EST project" EMBL DATABASE ENTRY MMA44549, ACCESSION NUMBER AA044549, 6 September 1996, XP002073850 see the whole document ---	1,2
X	M. MARRA ET AL: "The WashU-HHMI mouse EST project " EMBL DATABASE ENTRY MM24137, ACCESSION NUMBER W83241, 28 June 1996, XP002104301 cited in the application see the whole document ---	1,2
-/--		



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

31 May 1999

Date of mailing of the international search report

11/06/1999

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/00513

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. MARRA ET AL: "The WashU-HHMI mouse EST project" EMBL DATABASE ENTRY MM10142, ACCESSION NUMBER W87101, 2 July 1996, XP002104302 see the whole document ----	1,2
A	US 5 449.758 A (J.L. HARTLEY, FREDERICK, MD.) 12 September 1995 cited in the application see claims ----	11,12
A	YAO Z ET AL: "HUMAN IL-17: A NOVEL CYTOKINE DERIVED FROM T CELLS" JOURNAL OF IMMUNOLOGY, vol. 155, no. 12, 15 December 1995, pages 5483-5486, XP000602481 cited in the application see the whole document ----	1-13
P,X	WO 98 49310 A (ZYMOGENETICS, INC.) 5 November 1998 see the whole document especially sequences ID no.1, 2, 11, 12, 39 and 40 see example 1 see claims -----	1-10,13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/00513

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5449758	A	12-09-1995	NONE	
WO 9849310	A	05-11-1998	AU 7152798 A	24-11-1998